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INTRODUCTION

Magnetic particles with micron or sub-micron dimensions are now becoming highly attractive for many biomedical applications, such as targeted drug delivery, gene therapy, disease detection, biochemical sensing, genetic screening. Advances in these areas are largely due to the research progress in nano-technology and in magnetic fluid technology, in particular. The last one gave birth to the magnetic fluid hyperthermia (MFH) - an important tool for cancer treatment.

Hyperthermia is a cancer therapy consisting of heating a tumor region to the elevated temperatures in the range of 42-45 ^oC for an extended period of time (2-8 hours). This leads to thermal inactivation of cell regulatory and growth processes. Moreover, heat boosts the tumor response to other treatments such as radiation, chemotherapy or immunotherapy. Of particular importance is careful control of generated heat in the treated region and keeping it localized. Higher heating, to about 56^o C can lead to a healthy tissue thermo-ablation. With accurate temperature control, hyperthermia has the advantage of having minimal side effects. Several heating techniques are utilized for this purpose, such as whole body hyperthermia, fever induced hyperthermia, radio-frequency (RF) hyperthermia, ultrasound technique, microwave hyperthermia, inductive needles (thermo-seeds), and magnetic fluid hyperthermia (MFH).

Magnetic fluid hyperthermia offers many advantages such as targeting capability by applying magnets. However, this technology still suffers significant inefficiencies due to lack of thermal control. Nano-particles with inherent thermoregulating properties may solve this problem. The concept of thermoregulating particles for hyperthermia is an approach where an alternating magnetic field is used to excite magnetic particles and generate heat. It is novel in that it has a built-in safety switch – a low Curie temperature (temperature at which magnetic properties are lost) that limits the maximum temperature. Although there are known many magnetic systems with the desired Curie temperature (43^oC), a number of additional requirements has to be fulfilled. This includes non-toxicity, fast removal from the organism, high absorption of RF power, good targeting capabilities, good MRI contrast. In order to develop a nano-material, competitive to the present state-of-the-art Fe_2O_3, a significant amount of research is needed in several areas, such as nano-fabrication technology, physics of magnetism, numerical thermal modeling, MRI thermo-tomography, toxicology, medical trials, etc.

The research goal of this project is to develop a novel class of magnetic fluids that exhibit thermo-regulating properties attractive for a number of medical applications. These fluids could potentially surpass the functionality of currently available magnetic fluids used in hyperthermia. The specific goals are:

1. Develop a synthesis process to fabricate magnetic nano-particles with adequate characteristics for thermo-regulating magnetic fluid (TRMF) operating in 40-45^o C range.

2. Test and characterize TRMF using microscopy, diffraction and spectroscopy techniques such as powder XRD diffraction, dynamic light scattering, FTIR and HRTEM, SAR measurements, magnetic susceptibility measurements, etc.
3. Develop a thermal breast model with the distributed TRMF and determine the volumetric thermal field characteristics in the presence of RF heating.
4. Evaluate the performance and advantages of the TRMF as compared to the ordinary MF.
5. Evaluate the efficacy of thermo-regulating magnetic fluid in breast cancer hyperthermia.

LITERATURE UPDATE ON THERMOREGULATING NANO-PARTICLES

Since the commencement for this research project, new progress on thermoregulating nano-particles has been reported in literature. Complex systems based on $Mn_xZn_y[Fe_{2-x}Gd_z]O_4$ and $Mn_xZn_yFe_2O_4$ have been evaluated for possible hyperthermia applications [1]. The Curie point of all the synthesized compositions proved to be too high for the auto-regulation of temperature in the desirable range. Apparently, it was not possible to get the ferrites of this composition with the Curie point lower than 70–100 ^oC. A number of ferrites: $ZnFe_2O_4$ (T_C = 100 - 102°C), $La_{0.8}Sr_{0.2}MnO_3$ (T_C = 48°C) and $La_{0.75}Sr_{0.25}MnO_3$ (T_C = 56°C) have been prepared by the freeze-drying synthesis technique and the RF absorption rate has been measured. No promising results were obtained [2]. A significant number of various systems has been synthesized and characterized by V. Mohite from the Florida State University in 2004 as her thesis work. Ferrites of the following systems: Fe-Zn-Mn , Fe-Gd-Zn-Mn, Mn-Zn-Gd, Fe-Zn have been obtained. Also, Gd_4C, Ni-Cu and Fe-Gd-B particles were synthesized. This preliminary work suggests that some gadolinium containing systems could be promising as they could yield material with the desired Curie temperature. Gadolinium-based compounds: $Gd_5(Si_{1-x}Ge_x)_4$, $(Gd_{1-x}Ho_x)_5Si_4$ have been synthesized and magnetization was measured in [3]. Cu-Ni based nanoparticles have been reported as potential candidates for hyperthermia developments [4].

SYNTHESIS WORK

Our synthesis and characterization efforts concerning nickel-based alloys have been reported previously [5]. Nano-material has been obtained using an inverse micelle method at the Ohio State University (OSU). Samples of the material have been mailed to the MSFC laboratory where the magnetic properties were measured by the vibrational sample magnetometer (VSM). All the samples displayed very small magnetization. This is believed indicative of oxidation of nickel-based particles. The material has then been analyzed at the OSU right after the synthesis. It displayed the expected magnetic behavior of non-oxidized material. We came to the conclusion that the material is slowly oxidizing (within a few days). A few recent publications on nickel nano-material confirmed our observations [6]. However, sparse literature exists on the subject of chemical stability of the nano-particles.

Although metal alloys may be more efficient in terms of heating efficiency as compared to ferrites, they are difficult to protect against oxidation [7]. Recent study of iron nano-particles coated with gold nano-shells indicated that the layer dose not prevent against oxidation [8]. Apparently, the gold nano-layer is not continuous. There is one report from India on gold coating using glucose as a novel reduction medium [9]. However, no data on nano-layer properties of this novel method is given. Note, that gold surface is very attractive for bio-technological functionalizing processing as one of the best for attachments of various ligands. However, difficulty of using the existing methods of gold coating is apparent.

In view of the above recent research papers on nano-Ni and gold nano-shells, we have expanded our research field to include some additional potentially promising methods and systems. Two novel methods of synthesis of nickel-based nano-particles have been included into our research project. Specifically, we are investigating the polyol synthesis method, and the method based on thermal decomposition of carbonyls and acetylacetonates in high temperature boiling ethers. These methods have the advantage of growing larger amounts of particles, have a good size control, particles are made at higher temperature, so that the crystallinity is improved, particles can be easily capped with oleates and possibly with gold. Optical characterization for analysis of nano-particle shell strucrute/properties is being conducted now (see the attached technical report from SSS Optical Technologies LLC).

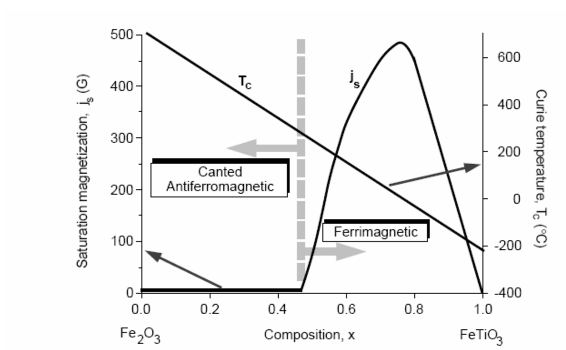


Fig.1. Magnetization of Fe-Ti oxides as a function of composition

Fe-Ti minerals are biocompatible materials and promising candidates for thermoregulating nano-particles (see Fig.1). Dr. Kosacki from Oak Ridge National Laboratories (ORNL) has performed a series of synthesis of this material by a proprietary polymeric method. The obtained nano-material exhibits good size uniformity (5% deviation)(see Fig.2). Several (six) vacuum/nitrogen annealed and non-annealed samples were prepared at ORNL and analyzed at MSFC/NASA. Fig.3. displays the hysteresis curve for one of the samples. The magnetization as a function of temperature is displayed in Fig.4.

Curie temperature lower than approx. 200^{o}C was not obtained. It appears that the required composition is metastable and difficult to obtain in the nano-size form.

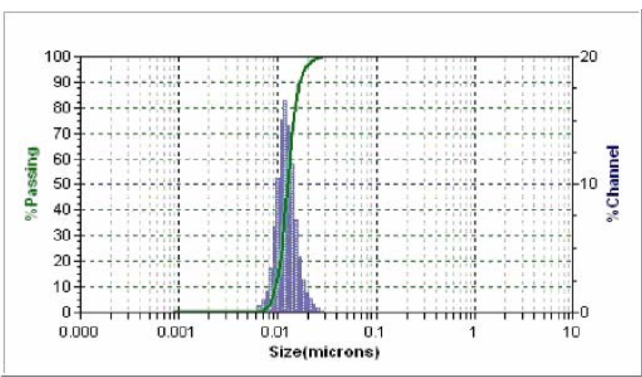
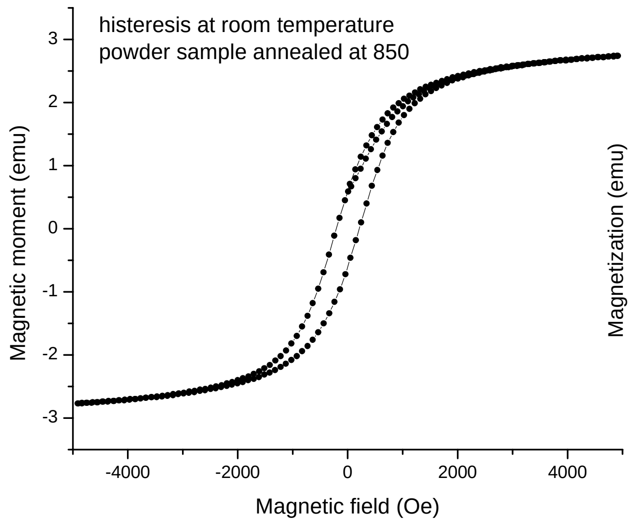


Fig.2. Size distribution of Fe-Ti oxides made at ORNL labs. The average size is 12nm.



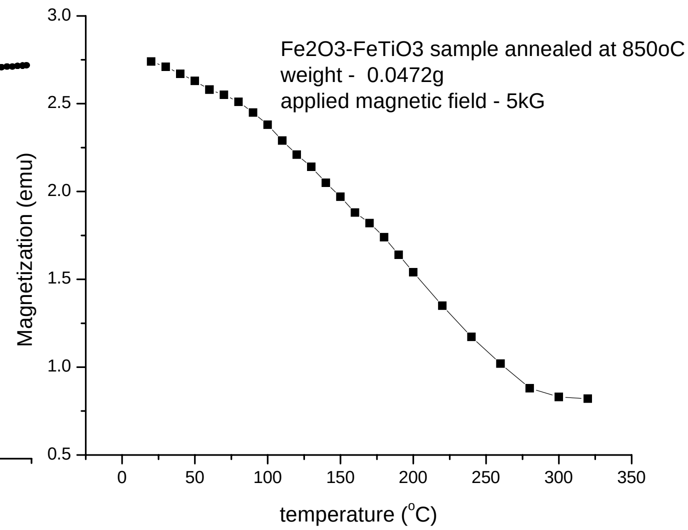


Fig.3. Hysteresis curve for Fe-Ti ferrites

Fig.4. Magnetization of Fe-Ti ferrite as a function of temperature

We have investigated Mg-Ti-Fe-O spinels that could be attractive for hyperthermia. This work was a natural extension of our study on Fe-Ti-O system. Dr Matsuki from Japan has proposed thermo-regulating thermo-seeds based on spinels that could have a required 43^{o}C Curie temnperature[10]. This material is non-toxic and potentially could be used for our work. We have synthesized the nanoparticles by sintering MgO, FeO, TiO at high temperature of 1300^{o}C and then ball milled in water with surfactant (CTAB) for three weeks. The nano-powder exhibits the required magnetic behavior. The magnetization of the nanopowder is depicted in Fig.5 and its Curie point is 55^{o}C. As it can be seen from this figure, the magnetization drops down slowly. Depending on the applied RF power level, the temperature could reach different values. Fig. 6 depicts the temperature change of the nano-particle suspension in water for the most attractive power level. This nano-material is planned to be chemically synthesized and characterized as one of the most promising thermoregulating nanoparticles investigated so far.

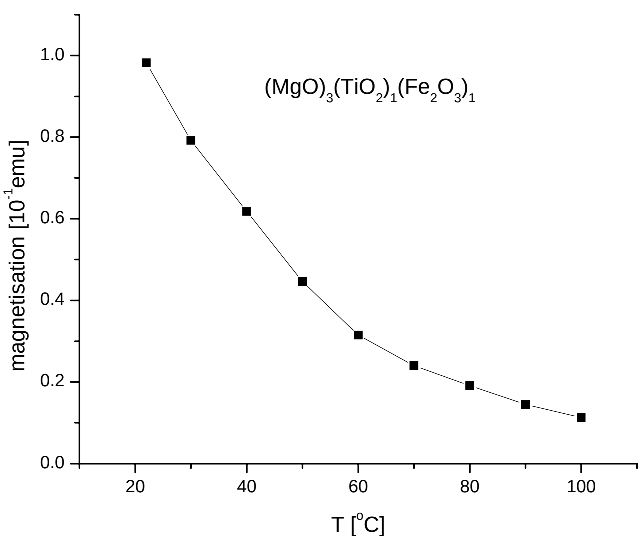


Fig.5. Magnetization of Mg-Ti-Fe spinel nanomaterial.

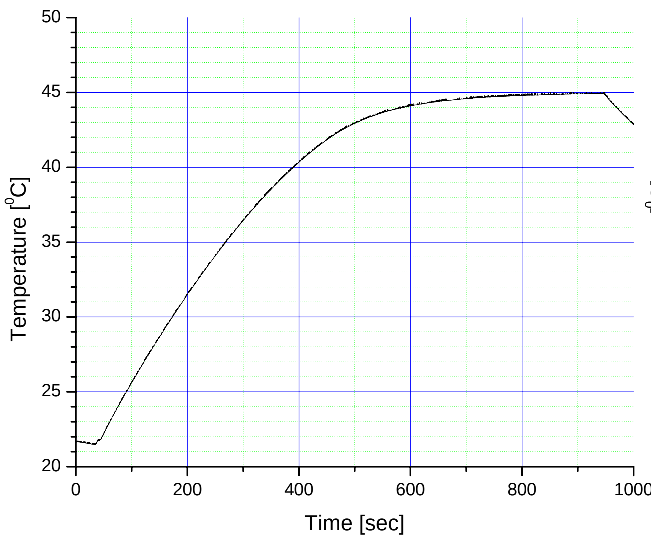


Fig. 6. The heating curve of the Mg-Fe-Ti spinel nanoparticles dispersed in water displaying the required for hyperthermia saturation behavior at 45^{o}C.

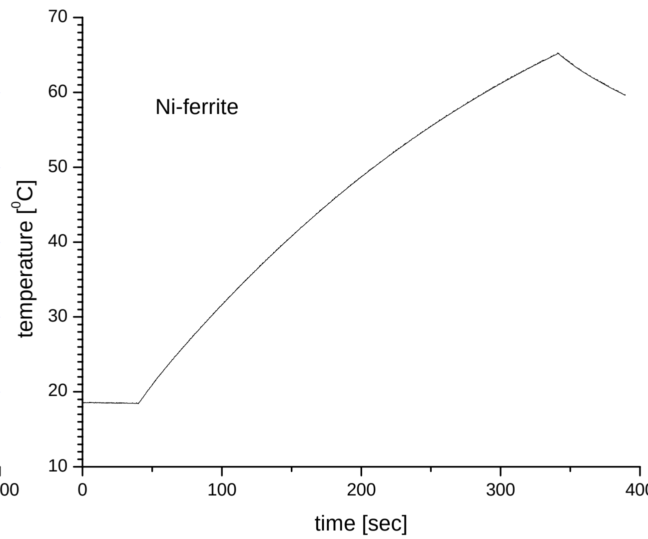


Fig.7. Typical heating curve for non-selfregulating magnetic nanoparticles of nickel ferrite with T_C of 160^{o}C.

The task on the reverse micelle method, developed at OSU, has been completed and reported in the previous year. The related characterization work was completed. After difficulties with oxidation, we have attempted to synthesize the proposed nickel-based material by recently proposed polyol method [11]. The capping of the particles can be achieved by the oleic acid and oleylamine during synthesis. Nickel nanoparticles have been successfully grown during two runs.

We have also synthesized nano-particles of magnetite by several methods. The material has been used for initial development of the optical characterization methods being developed (see the attached separate report). We have successfully synthesized nano-particles of iron that are stable in air, alcohol and water. The method consisted of a thermal decomposition of pentacarbonyl iron in dioctyl ether at 200^{o}C with ammonia/nitorgen bubbling thru the fluid. The obtained nano-powder is highly magnetic and it is presumably coated with the nano-layer of iron nitride. Note, that pure iron has one of the record high magnetization. However, because of its oxidation problems, has never been investigated for hyperthermia. The discovered synthesis route is potentially useful for nickel-based nano-particles, proposed for this project.

MODELING WORK

In order to theoretically demonstrate the advantage of the novel magnetic nanoparticles for hyperthermia, we have developed a thermal model of a breast tumor MH. The model is based on the bioheat equation proposed by Pennes:

$$\rho c \frac{\partial T}{\partial t} = \nabla (k \nabla T) - c_b w (T - T_b) + A + Q$$

where c - is specific heat,
κ –thermal conductivity,
w –blood perfusion rate,
A- metabolic heat rate,
Q-electromagnetically induced heat rate,
T_b-blood temperature,
c_b-specific heat of blood,
ρ - tissue density.

$$\frac{k}{\rho c} = 1.381 \times 10^{-7}\, m^2/s$$

$$\frac{A}{\rho c} = 1.326 \times 10^{-4} Hz$$

$$q = \frac{Q}{\rho c} = \frac{0.2}{e^{(T-40)} + 1} e^{-0.1 r^2}$$

$$\frac{c_b w}{\rho c} = \begin{cases} 9.66 \times 10^{-4} \left(0.45 + 3.55 e^{-(T-45)^2/12}\right) Hz \\ 5.1 \times 10^{-4} Hz \end{cases}$$

$$T_b = 37\,^0C$$

Note that the bio-heat equation is non-linear: blood perfusion and heat generation are temperature dependent.

Fig.8 displays the geometry of the modeling system. Approximately 10^4 cells were used for this calculation. Fig. 9 displays the temperature distribution along the z-axis after reaching a stationary state for several levels of radio frequency power. Red curves correspond to thermo-regulating particles, and black curves correspond to the ordinary nano-particles. Finally, Fig. 10 represents the temperature at the center of the tumor for various excitation power levels for two types of magnetic particles. Significantly better temperature control can be achieved by thermo-regulating particles.

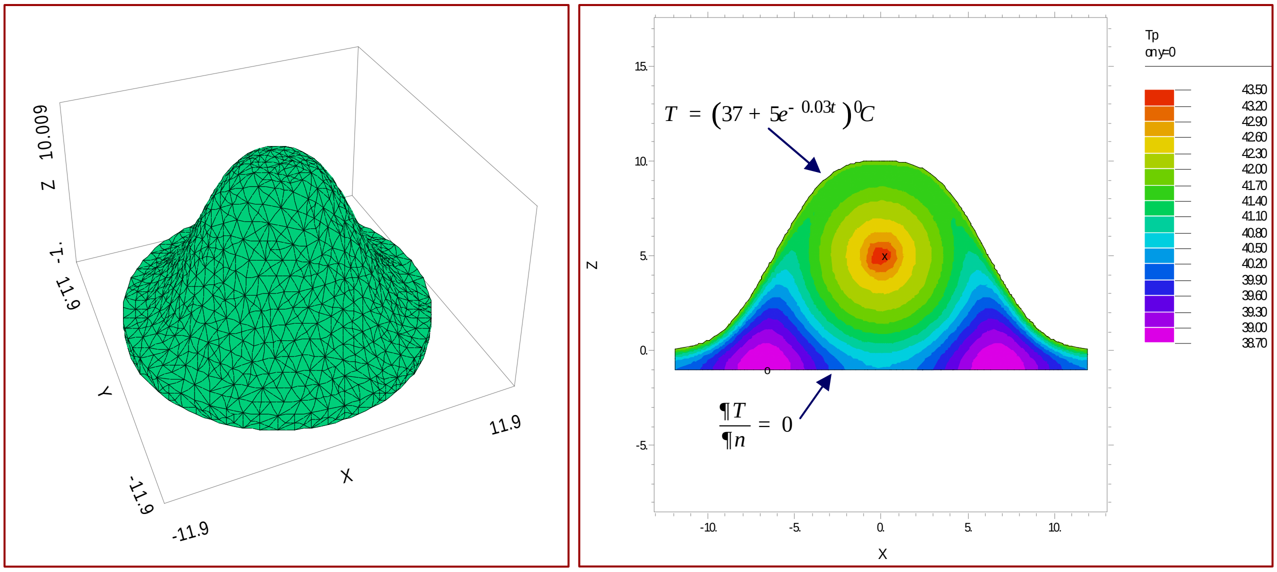


Fig.8. Geometry of the 3-D model of the breast tumor. On the right: stationary temperature distribution on (y=0) surface.Tumor area is 2 cm diameter centered at the position (0,0,5). The magnetic particles distribution is centered at the same point.

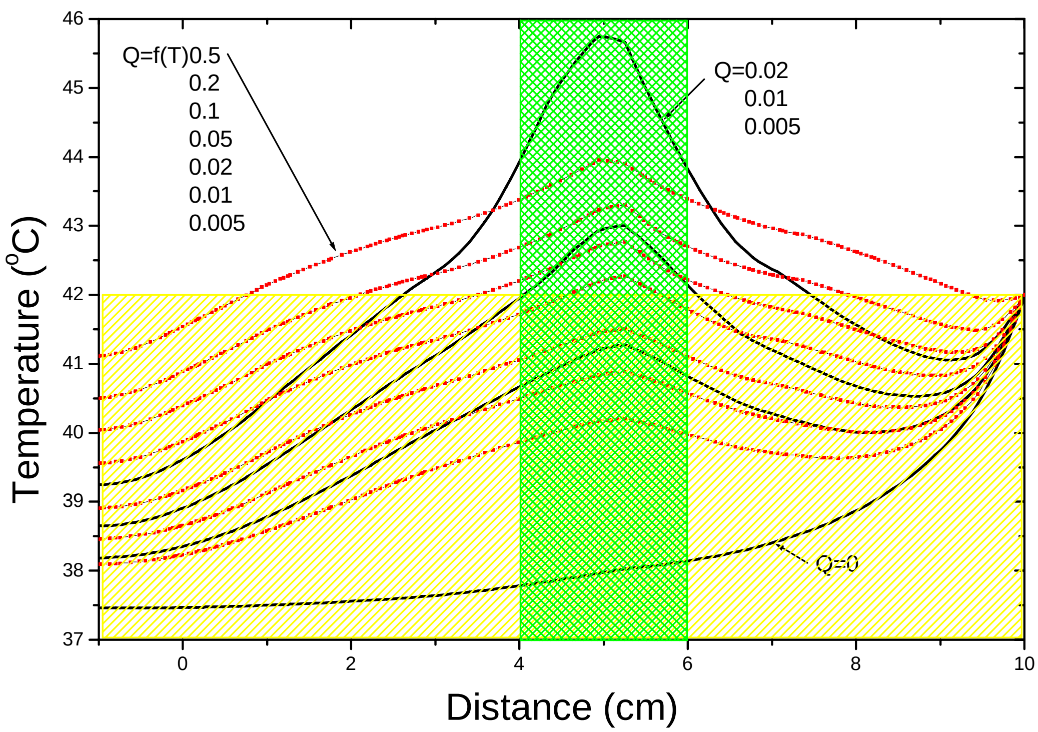


Fig.9. Temperature distribution along the central line for different levels of RF excitation. Red curves correspond to self-regulating particles while black are for ordinary material.

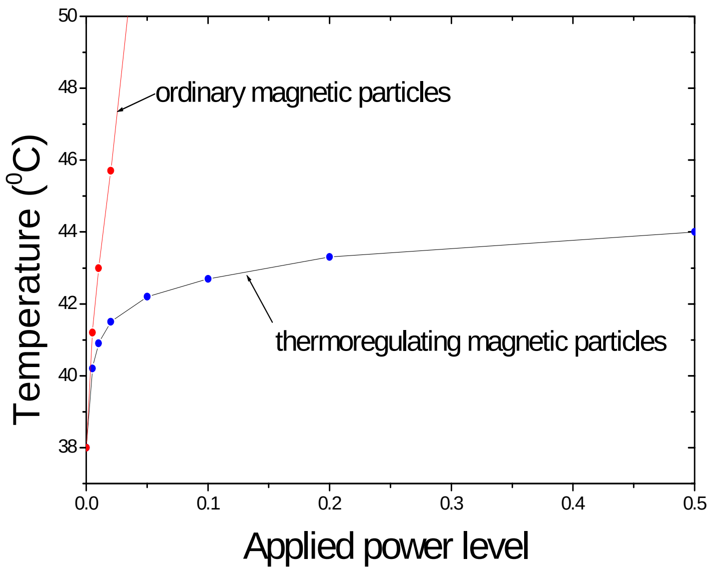


Fig.10. Temperature control improvement displayed by novel particles.

RF HEATING WORK

A year ago, the MSFC inductive heating unit became inaccessible to the PI. The home-made RF power system has been built and is being used now for this work. The frequency range of the system is 100 - 450 kHz and the power is 3kW. Figs. 6 and 7 display the heating curves of the nano-particles suspension in water. The SAR test measurements are being conducted now. The experimental system for the measurements of the thermal response breast tissue model has been fabricated. It consists of an agar gel enclosed into the hemispherical container and a small volume of the dispersed nano-particles at the center of the container. The RF heating will be recorded in different positions by the embedded into agar six thermistors. The magnetite vs. thermo-regulating nanoparticles will be compared to validate the thermo-regulating concept. The experimental data will be confronted with the theoretical modeling assuming no blood perfusion (agar). This experimental work is needed to complement the theoretical work explained above briefly.

CHARACTERIZATION

This task regularly accompanies the synthesis work. Characterization analysis includes size, composition, magnetic properties, and RF heating properties. The available XRD system is being used for size, and composition analysis. Magnetic measurement are done by the VSM system that give information about static magnetic properties of the nano-powder. We have realized that state-of-the-art optical characterization can provide valuable information about the size and surface properties of the nano-colloids. This information is critical in assessing the oxidation and capping phenomena. We have recently acquired an optical dynamic scattering system that could give the hydrodynamic

size distribution of the nano-particles. This graph can be obtained in a few minutes, allowing almost in-situ monitoring the syntyhesis progress. UV-Vis absorption and FTIR spectroscopy has been also investigated as a valuable characterization tool. Recently, atomic force microscopy became available, that gives useful information about the size and shape of the nanoparticles.

CONCLUSION

A number of potential systems to produce thermo-regulating magnetic nano-particles has been investigated:

1)The Pt-Ni nano-alloy has been synthesized in the micellar medium. This nano-material, when annealed in a reducing atmosphere, displays predicted magnetic properties. Gold coating of these nanoparticles against oxidizing in air appears problematic.

2)Fe-Ti-O ferrite. Nano-particles of this material have been synthesized in ORNL. They display high magnetization with the Curie temperature above 200^0C.

3)Mg-Ti-Fe-O ferrite. This is an attractive biocompatible system. Nano-powders have been fabricated by sintering and ball-milling. They exhibit high magnetization and a good control of the Curie temperature within the 30-60^oC range. RF heating of the suspension exhibits expected thermo-regulating property.

Modeling work on breast magnetic fluid hyperthermia has demonstrated that thermo-regulating magnetic fluids offer significant advantages over the ordinary magnetic fluids. New systems have been included into the project, when the oxidation issue became apparent. However, it can be due to the particular synthesis route. Therefore, we have not abandon nickel based systems, but tested two novel synthesis methods that could be technologically advantageous. New characterization techniques based on optical properties are included into this research as well.

The task schedule for the next reporting period does not require changes, as the thermo-regulating magnetic fluid needed for further work has been fabricated.

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